Title: New Insights on Profiling of Phorbol, Deoxyphorbol, Ingenol and Jatrophane Diterpene Esters by High Performance Liquid Chromatography Coupled to Multiple Stage Mass Spectrometry

Author: Louis-Félix Nothias-Scaglia Isabelle Schmitz-Afonso Franck Renucci Fanny Roussi David Touboul Jean Costa Marc Litaudon Julien Paolini

PII: S0021-9673(15)01444-2
DOI: http://dx.doi.org/doi:10.1016/j.chroma.2015.09.092
Reference: CHROMA 356915

To appear in: *Journal of Chromatography A*

Received date: 9-6-2015
Revised date: 7-9-2015
Accepted date: 30-9-2015

Please cite this article as: L.-F. Nothias-Scaglia, I. Schmitz-Afonso, F. Renucci, F. Roussi, D. Touboul, J. Costa, M. Litaudon, J. Paolini, New Insights on Profiling of Phorbol, Deoxyphorbol, Ingenol and Jatrophane Diterpene Esters by High Performance Liquid Chromatography Coupled to Multiple Stage Mass Spectrometry, *Journal of Chromatography A* (2015), http://dx.doi.org/10.1016/j.chroma.2015.09.092

New Insights on Profiling of Phorbol, Deoxyphorbol, Ingenol and Jatrophane Diterpene Esters by High Performance Liquid Chromatography Coupled to Multiple Stage Mass Spectrometry

Louis-Félix Nothias-Scaglia[a,b], Isabelle Schmitz-Afonso[b,‡], Franck Renucci[a], Fanny Roussi[b], David Touboul[b], Jean Costa[a], Marc Litaudon[b*]marc.litaudon@cnrs.fr, Julien Paolini[b*]paolini@univ-corse.fr

[a]Laboratoire de Chimie de Produits Naturels, UMR CNRS SPE 6134, University of Corsica, 20250, Corte, France
[b]Institut de Chimie des Substances Naturelles ICSN-CNRS UPR 2301, LabEx CEBA, Univ. Paris-Sud, 1 avenue de la terrasse, 91198, Gif-sur-Yvette, France
*Corresponding authors. Tel: + 33 1 69 82 30 85, fax: + 33 1 69 07 72 47

Abstract
This paper reports our effort to develop a comprehensive HPLC-MS^n-based dereplication strategy for phorbol ester (PE), deoxyphorbol ester (DE) and ingenol ester (IE) profiling in plant extracts. This strategy is composed of two sequential analysis exploiting specific hybrid triple quadrupole/linear ion trap instrument modes. A first run was performed using a multiple reaction monitoring (MRM) mode targeting fragmentation of PE and DE/IE coupled with the acquisition of MS^2 spectrum for the ions at *m/z* 311 and *m/z* 313, respectively. A second run was then completed based on precursor ion scan mode (PIS) and automatic MS^2 acquisition for each pseudo-molecular ion. The developed approach was used to investigate ten *Euphorbia* extracts showing bioactivity against chikungunya virus replication. Experiments allowed partial annotation of three DE/IE but no PE was detected. Results suggested that other types of diterpene esters displayed PE- and DE/IE-like fragmentations. The study of jatrophane ester (JE) standards by CID fragmentation using low and high resolution mass spectrometry confirmed this hypothesis, highlighting challenges and difficulties of diterpene esters profiling within plant extracts. Nonetheless, the present LC-MS^n method can be easily adapted to profile other types of diterpene esters.

Keywords
Phorbol, Deoxyphorbol, Ingenol, Jatrophane, Diterpene Esters, HPLC-MS^n

1. Introduction

Within natural products chemistry, diterpene esters from Euphorbiaceae species represent a unique group of structurally highly diverse compounds [1–4]. From *Euphorbia* species, over 550 isolated diterpene esters, incorporating more than 20 skeletal types, have been isolated up to date [5]. In an effort to identify novel inhibitors of CHIKV (chikungunya virus) replication, we recently found that *Euphorbia* extracts displayed potent anti-CHIKV activity [6]. Diterpene esters such as tigliane, ingenane, daphnane and jatrophane-types were found to be potent and selective inhibitors of CHIKV replication [7–11]. In particular, tigliane-type diterpenes, such as TPA (12-*O*-tetradecanoylphorbol-13-acetate) and phorbol-12,13-didecanoate were found to display potent anti-CHIKV activity [11], but are also known to display strong tumor-promoting and pro-inflammatory activities [12–14] by their ability to modulate PKC (protein kinases isoenzymes) [15].

In order to develop a dereplication method, a targeted High Performance Liquid Chromatography coupled to multiple stage Mass Spectrometry (HPLC-MS^n) method was developed and applied for the screening of twenty-nine reference standards of diterpene esters in *Euphorbia* extracts displaying anti-CHIKV activity [6,8]. By this mean, no known anti-CHIKV compound could be detected in antiviral *Euphorbia* extracts.